Detoxification and Tolerance of Heavy Metal in Tobacco Plants

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Abstract The higher content of heavy metals in tobacco (*Nicotiana tabacum*) not only affects its growth, development, and economic benefit, but also threatens public health fatally. This chapter reviews the physiological detoxication mechanism of tobacco on heavy metals (HMs), such as the transport, accumulation, and compartmentalization of HMs in tobacco, the research progress in effects of HMs on tobacco antioxidative enzymes and antioxidants activity, the role of trichomes and crystals, and the special genes influencing tobacco HMs accumulation. We also put forward some integrated approaches for preventing heavy metal pollution of tobacco or improving polluted soil remediation efficiency.

Keywords Tobacco · Heavy metal · Phytoremediation · Transgenic tobacco · Heavy metal accumulation

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Contents

1 Introduction

Increased industrialization, urbanization, and anthropogenic activities have enhanced the levels of metal pollutants in the environment. Disposal of inorganic metalliferous wastes, accidental and process spillages, inadequate residue disposal, mining and smelting of ores, and heavy metal contaminated sewage sludge application to agricultural soils are responsible for the migration of metals adding pollution to our ecosystem (Ghosh and Singh 2005; Kavamura and Esposito 2010; Chatterjee et al. 2013). Heavy metals (HM), which in turn enter the food chains, cause damage to life-forms of different trophic levels. However, being sessile in nature, plants cannot migrate from one place to other to avoid natural or edaphic stress factors like HM. Therefore, plants extract ions from the soil and concentrate them in their tissues leading to elevated concentrations of HM in the biomass of plants growing in HM contaminated soils. This ability of plants can be used in HM phytoextraction, when metal-accumulating plants are grown on a contaminated site and HMs are removed within their harvestable parts (Macek et al. 2004; Janouskova et al. 2005). Tobacco (*Nicotiana tabacum*) is a widely used plant model for growth in heavy metal contaminated sites. Its high biomass and deep rooting system makes it interesting for phytoextraction. Tobacco is one of the basic agricultural products in the world, with social and economic importance. Tobacco easily accumulates certain heavy metals, particularly cadmium (Cd) in leaves (Table 1).

When grown for energy production instead of for smoking, tobacco can generate a large amount of inexpensive biomass more efficiently than almost any other agricultural crop. Tobacco possesses potent oil biosynthesis machinery and can accumulate up to 40 % of seed weight in oil. In the search for alternative biofuel plant resources, tobacco has been largely overlooked being considered primarily an expensive crop grown for smoking. However, closer examination identifies tobacco, which is cultivated in more than 100 countries worldwide, as an

Table 1 List of genes influencing heavy metals accumulation in tobacco

Genes	Function	Source of genes	Medium	Results	References
BjPIP1	An aquaporin cDNA	*Brassica juncea* L.	MS agar plates half-strength Hoagland solution	Overexpression of *BjPIP1* in tobacco enhanced Cd resistance of root growth. Moreover, the *BjPIP1* overexpressing plants showed higher activities of antioxidative enzymes, and lower level of electrolyte leakage and MDA content under Cd stress	Mol Biotechnol (2008) 40
AtPCS1 CePCS	Phytochelatin synthase	*Schizosaccharomyces pombe*	Hydroponic conditions	Plants overexpressing *AtPCS1* were Cd-hypersensitive although there was no substantial difference in cadmium accumulation between studied lines. A dramatic accumulation of γ-glutamylcysteine and concomitant strong depletion of glutathione	J Exp Bot (2008) 59
tzn1	Zinc transporter gene	*Neurospora crassa*	Hydroponic Hoagland's medium	Transgenic plants showed enhanced accumulation of Zn (up to 11 times) compared to control plants	Plant Cell Environ (2010) 33
MuSI	Dehydration the rubber elongation factor	Sweet potato	Yamazaki solution	Cd translocation from roots to shoots was reduced in the transgenic plants, thereby avoiding Cd toxicity	Plant Biotechnol Reps (2011) 5
TvGST	Glutathione transferase	*Trichoderma virens*	Hydroponic conditions	More tolerant to Cd, without enhancing its accumulation in the plant biomass	PLoS ONE (2011) 6
tcu-1	High-affinity copper transporter gene	*Neurospora crassa*	Hoagland's solution and soil condition	Higher acquisition of Cu (up to 3.1 times) compared with control plant shoots and roots showing 1.33 and 1.64 times more Cu accumulation	Plant Cell Rep (2011) 30

(continued)

Table 1 (continued)

Genes	Function	Source of genes	Medium	Results	References
AtECA3	Encoding a P2A-ATPase	*Arabidopsis thaliana*	Hydroponic conditions	Better growth of plants at moderate levels of Mn (2 μM) and enhanced tolerance to high Mn (100 μM)	Environ Exp Bot 202–209 (2011) 72
MT2b HMA4	*Arabidopsis* metallothionein HMs transporting ATPase	*Arabidopsis thaliana*	Half-strength Hoagland's solution	The double transformant exhibited enhanced Cd-tolerance, enhanced Cd and Zn root to shoot transport, but unaltered Zn tolerance and Cd and Zn uptake, compared with wild type	Environ Exp Bot 71–76 (2011) 72
EhMT1	Metallothionein	*Elsholtzia haichowensis* Sun	Hoagland solution	In the presence of 20 μM Cu, the shoots and roots of transgenic plants had significantly higher total Cu contents compared to WT plants. The shoot/root ratio of the Cu content in transgenic plants was lower than that in WT plants	J Hazard Mater (2012) 233

outstanding industrial biomass crop, which can generate up to 170 tons ha^{-1} of green tissues when grown for biomass production instead of for smoking (Schillberg et al. 2003). Moreover, like hardwood trees, tobacco can be coppiced to stimulate resprouting from the stump after cutting; thus, multiple biomass harvests are possible in a single year.

2 Distribution Rule of HMs in Tobacco

Experiment on hydroponic seedlings was carried out to study the subcellular distribution and chemical forms of Cd in the root system and leaves of two tobacco genotypes (K326 and *N. rustica*) with different Cd accumulation patterns at different Cd treatment levels (1 and 50 μmol L^{-1}). At low level, Cd accumulated in root system and leaves of K326, while it accumulated mainly in the root system of *N. rustica*. At high level, Cd mainly accumulated in the root system of both varieties. Cd mainly bound to the soluble fraction in both varieties at low level, which accounted for about 70 % of the total Cd content. The Cd retention by cell wall increased significantly in K326 at high Cd level. The binding proportion of Cd in soluble fraction is always identical to the Cd content in the respective parts, which indicates that the difference in binding ability in soluble fraction might be one of the major factors affecting Cd accumulation in the two genotypes. No significant difference was observed between the chemical forms of Cd in the two genotypes, indicating that Cd accumulation in tobacco was not relevant to its chemical form (Tian et al. 2012).

Chen et al. (2007) found that the vital parts accumulating Cd, Pb in plant was leaf and the sensitive parts were polluted by Cd, Pb; the subsidiary sensitive parts were polluted by Cr, Ni. Heavy metal in organic manure did not lead to accumulation of heavy metal in the plant; the content of Pb, Cd, and Cr is less than the disposal of the pure fertilizer; the disposal of 7 kg chemical nitrogen with 1 kg organic nitrogen is more favorable to inhibiting the accumulation and distribution of As, Cd, Cr, Pb, Hg in tobacco leaves.

3 Physiological Detoxification Mechanism of Tobacco to HMs Stress

3.1 *Antioxidative Enzymes or Antioxidants*

Most tobacco growing region soils contain excessive Cd^{2+} and Pb^{2+}. Pot experiments carried out in the field revealed that the contents of both Cd^{2+} and Pb^{2+} in roots were significantly increased along with stress time and the amounts of Cd^{2+} and Pb^{2+} added to soil. The growing of tobacco in Cd^{2+} and $Cd^{2+} + Pb^{2+}$ polluted soil for 50, 100, and 150 d resulted in abnormal external morphological and anatomical changes in the ripe region of lateral roots. All the abnormal roots had abnormal vascular cylinders, and the ratio of abnormal external morphological and anatomical changes in roots positively correlated with the Cd^{2+} contents in roots and stress time; while there were no abnormal external morphological and anatomical changes in roots under Pb^{2+} stress. It was suggested that Cd^{2+} stress caused abnormal anatomic changes in roots, but Pb^{2+} stress did not (Yuan et al. 2011). Kusaba et al. (1996) reported an auxin-regulated gene expressed in tobacco under Cd^{2+} stress, and this glycine-rich protein expressed in the plant vasculature. In this experiment the abnormal morphological and anatomical changes under Cd^{2+} and $Cd^{2+} + Pb^{2+}$ stress suggested that Cd^{2+} might induce some gene mutation or expression which might relate to programmed cell death, auxin content, and auxin transportation, while Pb^{2+} might not induce these gene mutations or expressions, hence under single Pb^{2+} stress there were no abnormal morphological and anatomic changes (Yuan et al. 2011). Nevertheless, changes of all parameters depend on the severity and duration of metal stress and plant species. Root and shoot growth, net photosynthetic rate, and stomatal conductance were significantly reduced in plants treated with 100, 300, and 500 μM Pb $(NO_3)_2$. In plants treated with 500 μM Pb $(NO_3)_2$, the majority of stomata were closed. The effect of Pb $(NO_3)_2$ on chlorophyll content and chlorophyll fluorescence parameters was negligible. However, in plants exposed to 100, 300, and 500 μM Pb $(NO_3)_2$, the mesophyll cells showed altered chloroplasts with disrupted thylakoid membranes (Alkhatib et al. 2011).

Pot experiments indicated the malondialdehyde (MDA) content, protective enzyme activity, and cell membrane permeability of tobacco leaves; the cell membrane permeability and activity of tobacco roots decreased with the increase in Hg concentration. The chlorophyll content of tobacco leaves increased first and decreased afterwards; lower concentration of Hg (0.5 mg kg^{-1}) had a promoting effect on the chlorophyll content of tobacco leaves at rosette stage. The activity of catalas (CAT) was the most sensitive physiological index affecting the flue-cured tobacco leaves under Hg stress, which were in extreme negative correlation with the concentration of Hg (Cao et al. 2011). Biochemical investigations indicated restricted methylglyoxal accumulation and less lipid peroxidation under high zinc conditions in transgenic plants. Studies using the glutathione biosynthetic inhibitor, buthionine sulfoximine, suggested an increase in the level of phytochelatins and maintenance of glutathione homeostasis in transgenic plants during exposure to excess zinc as the possible mechanism behind this tolerance (Singla-Pareek et al. 2006).

In tobacco plants originating from different mutants grown under field conditions with varying fertilizer applications, the uptake of cadmium and zinc from soil increased with increasing biomass. Depending on Cd and Zn uptake, several antioxidant enzymes showed significantly different activities. Whereas SOD and CAT were usually elevated, several other enzymes and isoforms of GST were strongly inhibited. Heavy metal uptake represents severe stress to plants, and specific antioxidative enzymes are induced at the cost of more general reactions of the Halliwell–Asada cycle (Lyubenova et al. 2009). A novel DREB gene (*LbDREB*) from *Limonium bicolor* was cloned. Analysis of the role of *LbDREB* in tolerance to copper stress in transgenic tobacco showed that overexpression of *LbDREB* increased the contents of soluble protein and proline, and elevated the ratio of K to Na under $CuSO_4$ stress. Moreover, overexpression of *LbDREB* can upregulate stress-related genes that include Cu/Zn superoxide dismutase (Cu/Zn SOD), peroxidases (PODs), and lipid transfer proteins (LTP) (Ban et al. 2011). These results suggest that *LbDREB* can enhance plant copper tolerance by upregulating a series of stress-related genes, thereby mediating physiological processes associated with stress tolerance in plants.

The glyoxalase transgenics were able to grow, flower, and set seeds in the presence of 5 mM $ZnCl_2$ and sequestered excess zinc in roots. An increase in the level of PCs and maintenance of GSH homeostasis in transgenics during exposure to high $ZnCl_2$ levels seem to be the mechanisms behind this tolerance. The high accumulation of $ZnCl_2$ in the roots and the low accumulation of $ZnCl_2$ in the seeds of transgenic plants under high zinc conditions suggest the potential use of this engineering strategy in agriculture of crop plants in zinc-contaminated soil (Singla-Pareek et al. 2006). Cadmium stress at (100 μM) caused a significant inhibition of the growth of tobacco Bright Yellow-2 cells, and both proline and betaine significantly mitigated this inhibition. In addition, the mitigating effect of proline was more pronounced than that of betaine. Exogenous application of proline resulted in a decrease in lipid peroxidation and an increase in SOD and CAT activities without reducing Cd contents under Cd stress, while application of betaine resulted in a decrease in lipid peroxidation and an increase in CAT activity with reducing Cd accumulation (Islam et al. 2009). This study suggested that proline and betaine confer tolerance to Cd stress in tobacco BY-2 cells using different mechanisms.

To evaluate the functional roles of metallothionein (MT) in copper tolerance, we generated transgenic tobacco plants overexpressing *EhMT1* from the Cu-accumulator *Elsholtzia haichowensis* Sun. Overexpression of EhMT1 in tobacco plants imparted increased Cu tolerance based on seedling dry biomass when compared to wild-type plants. Plants expressing *EhMT1* accumulated more Cu in roots, which was mainly attributable to an increase in the soluble fraction. Levels of lipid peroxidation and production of hydrogen peroxide were lower in roots of transgenic tobacco than in wild-type plants. *EhMT1* was suggested to bind Cu in the cytoplasm, thereby decreasing activity of free Cu^{2+} ions and blocking Cu^{2+} from interacting with cytoplasmic components, which in turn decreases the production of reactive oxygen species. In addition, our results also indicate that *EhMT1*-overexpressing tobacco has a more efficient antioxidant system, with improved peroxidase activity to better cope with oxidative stress (Xia et al. 2012).

The 100 μM of Cd exposure reduced the total dry weight and chlorophyll index of the seedlings as much as the genuine Fe-deficiency. Concentration of Fe in the shoots decreased, whereas that in the roots increased due to Cd exposure, especially in the apoplasmic space. It is probable that Cd interferes mainly with the step of Fe-translocation from the roots to shoots and this sets the upper part of the plant in a state of Fe-deficiency. Cd exposure coordinately increased the expressions of the exogenous and endogenous Fe-deficiency responsive genes, *HvIDS2pro::GUS, NtFRO1,* and *NtIRT1* in the roots (Yoshihara et al. 2006). This is the first data to demonstrate the responses of Cd-inducible Fe-deficiency at a molecular level. Acetic, lactic, glycolic, malic, maleic, and succinic acids were found in tobacco and sunflower rhizosphere soils. Concentrations of LMWOAs increased with increasing amendment of Cd concentrations in tobacco and sunflower rhizosphere soils. The results suggest that the different levels of LMWOAs present in the rhizosphere soil play an important role in the solubilization of Cd that bind with soil particle into soil solution and then uptake by plants (Chiang et al. 2006).

3.2 The Role of Trichomes and Crystals

In tobacco, long and short trichomes can be distinguished morphologically. When tobacco seedlings were exposed to toxic levels of Cd, growth was retarded, but trichome number increased up to 2-fold in comparison with untreated samples. Observation by variable-pressure scanning electron microscopy (VP-SEM) indicated that large crystals of 150 μm in size were formed on head cells of both short and long trichomes. An energy dispersive X-ray analysis system fitted with VP-SEM revealed the crystals to contain amounts of Cd and Ca at much higher concentrations than in the head cells themselves. Transmission electron microscopy demonstrated crystal formation in amorphous osmiophilic deposits in vacuoles. When seedlings were treated with Cd in the presence of Ca, tolerance was increased in proportion to the increase in Ca concentration. These results indicate that tobacco plants actively exclude toxic Cd by forming and excreting Cd/Ca-containing crystals through the head cells of trichomes (Choi et al. 2001). Cations, such as Ca^{2+} and Mg^{2+}, are generally thought to alleviate toxicities of heavy metals through site-specific competition (Gupta et al. 2013).

Growth was severely inhibited when tobacco plants were exposed to toxic levels of cadmium (0.2 mM). However, when this treatment was combined with a high concentration of calcium (30 mM), the Cd-induced damage was strongly alleviated. Ca crystals were not only heavily deposited in the leaves but were also actively excreted from the trichomes, and both intra- and extracellular Ca crystals contained detectable amounts of Cd. Finally, Inductively Coupled Plasma Spectroscopy revealed that a high level of Ca (30 mM) suppressed Cd accumulation while also increasing the endogenous Ca concentration in the leaves. These observations imply that the amelioration of Ca against toxic Cd in tobacco plants is a result of not only the inhibition of Cd uptake, but also the extra- and intracellular sequestration of cadmium via Ca crystallization (Choi and Harada 2005). On the other hand tobacco trichomes play an important role in Cd crystal exudation through crystallization, but that, under NaCl stress, the long trichomes sequester those elements within their stalks (Choi et al. 2004). Almost 2,000 expressed sequence tag cDNA clones were sequenced to analyze gene expression in control and Cd treated tobacco leaf trichomes. RT-PCR analysis demonstrated that glutathione peroxidase and several classes of pathogenesis-related (PR) proteins were expressed specifically or dominantly in trichomes. The expression of osmotin and thaumatin-like proteins was induced by Cd treatment in both leaves and trichomes. Confocal laser scanning microscopy showed that glutathione levels in tip cells of both long and short trichomes were higher than those in other types of leaf cells, indicating the presence of an active sulfur-dependent protective system in trichomes (Harada et al. 2010).

4 The Special Genes Influencing Tobacco HMs Accumulation

The glyoxalase transgenics were able to grow, flower, and set normal viable seeds in the presence of 5 mM $ZnCl_2$ without any yield penalty. The endogenous ion content measurements revealed roots to be the major sink for excess zinc accumulation, with negligible amounts in seeds in transgenic plants. Preliminary observations suggest that glyoxalase overexpression could confer tolerance to other heavy metals, such as cadmium or lead. Comparison of relative tolerance capacities of transgenic plants, overexpressing either glyoxalase I or II individually or together in double transgenics, evaluated in terms of various critical parameters such as survival, growth, and yield, reflected double transgenics to perform better than either of the single-gene transformants (Singla-Pareek et al. 2006). A high affinity and high specificity zinc transporter gene (*tzn1*) from *Neurospora crassa* was cloned and introduced into *N. tabacum* with the objective of enhancing the potential of plants for zinc acquisition. When grown in hydroponic medium spiked with ^{65}Zn, transgenic plants showed enhanced accumulation of Zn (up to 11 times) compared to control plants, which was confirmed further by environmental scanning electron microscopy coupled with Energy Dispersive X-ray analysis. More importantly, no significant difference in uptake of Cd^{2+}, Fe^{2+}, Ni^{2+}, Cu^{2+}, Mn^{2+}, and Pb^{2+} between the transgenic and control plants was observed (Dixit et al. 2010). The present studies have shown that *N. crassa tzn1* is a potential candidate gene for developing transgenic plants for improving Zn uptake, without co-transport of Cd and may have implications in Zn phytofortification and phytoremediation.

With the objective of developing plants with improved copper acquisition, a high-affinity copper transporter gene (*tcu-1*) was cloned from fungus *N. crassa* and introduced into a model plant (*N. tabacum*). Transgenic tobacco plants (T0 and T1) expressing *tcu-1*, when grown in hydroponic medium spiked with different concentrations of copper, showed higher acquisition of copper (up to 3.1 times) compared with control plants. Transgenic plants grown in soil spiked with copper could also take up more copper compared with wild-type plants. Supplementation of other divalent cations such as Cd^{2+} and Zn^{2+} did not alter uptake of Cu by transgenic plants (Singh et al. 2011).

Wojas compared the effects of overexpression of *AtPCS1* and *CePCS* in tobacco (*N. tabacum* var. Xanthi), and demonstrated how the introduction of single homologous genes affects cellular metabolic pathways to a different extent leading to the opposite of the desired effect. In contrast to WT and *CePCS* transformants, plants overexpressing *AtPCS1* were Cd-hypersensitive although there was no substantial difference in cadmium accumulation between studied lines. In addition, PCS activity in *AtPCS1* transformants was around 5-fold higher than in *CePCS* and WT plants. *AtPCS1* expressing plants displayed a dramatic accumulation of γ-glutamylcysteine and concomitant strong depletion of glutathione. There was only a moderate and temporary increase in phytochelatin levels due to *AtPCS1* and *CePCS* expression. Marked changes in NPT composition due to *AtPCS1* expression led to moderately decreased Cd-detoxification capacity reflected by lower SH: Cd ratios, and to higher oxidative stress, which possibly explains the increase in Cd-sensitivity. The results indicate that contrasting responses to cadmium of plants overexpressing PCS genes might result from species-dependent differences in the activity of phytochelatin synthase produced by the transgenes (Wojas et al. 2008).

The transgenic tobacco exhibited a lower water loss rate, a decreased transpiration rate, and stomatal conductance compared to the wild-type plants under osmotic stress, indicating that *BjPIP1* might enhance plant drought resistance by decreasing transpiration via reducing stomatal conductance. Furthermore, overexpression of *BjPIP1* in tobacco enhanced Cd resistance of root growth, and lowered transpiration rate and stomatal conductance upon Cd exposure, suggesting that *BjPIP1* might increase heavy metal resistance by maintaining reasonable water status in tobacco. Moreover, the *BjPIP1*-overexpressing plants showed higher activities of antioxidative enzymes, and lower level of electrolyte leakage and malondialdehyde content under Cd stress, indicating *BjPIP1* might enhance the antioxidative activity and membrane integrity in transgenic plants. Taken together, these results suggest that *BjPIP1* might improve plant heavy metal resistance through alleviating water deficit and oxidative damage induced by metal ions (Zhang et al. 2008).

Less growth inhibition (higher tolerance) to all three metals was observed in 35S::*AtCAX2* and FS3::*AtCAX4* expressing plants. Consistent with the tolerance observed for Cd was the finding that while root tonoplast vesicle proton pump activities of control and *FS3AtCAX4* expressing plants grown in 3 μM Cd were similarly reduced, and vesicle proton leak was enhanced, root tonoplast vesicle antiporter activity of these plants remained elevated above that in controls. We suggest that CAX antiporters, unlike tonoplast proton pump and membrane integrity, are not negatively impacted by high Cd, and that supplementation of tonoplast with *AtCAX* compensates somewhat for reduced tonoplast proton pump and proton leak, and thereby results in sufficient vacuolar Cd sequestration to provide higher tolerance. Results are consistent with the view that *CAX2* and *CAX4* antiporters of tonoplast play a role in tolerance to high, toxic levels of Cd, Zn, and Mn in tobacco (Korenkov et al. 2007).

Since *MuSI* is upregulated in the roots of plants treated with cadmium or copper, the involvement of *MuSI* in cadmium tolerance was investigated in this study. *MuSI* transgenic plants were also more resistant to Cd. *MuSI* transgenic tobacco plants absorbed less Cd than wild-type plants. Cd translocation from roots to shoots was reduced in the transgenic plants, thereby avoiding Cd toxicity. The number of short trichomes in the leaves of wild-type tobacco plants was increased by Cd treatment, while this was unchanged in *MuSI* transgenic tobacco. These results suggest that *MuSI* transgenic tobacco plants have enhanced tolerance to Cd via reduced Cd uptake and/or increased Cd immobilization in the roots, resulting in less Cd translocation to the shoots (Kim et al. 2011).

5 How to Regulate Tobacco HMs Accumulation

5.1 Ensuring the Safety of Tobacco Leaf

The effect of arbuscular mycorrhiza (AM) on the phytoextraction efficiency of transgenic tobacco with increased ability to tolerate and accumulate cadmium (Cd) was tested in a pot experiment. Mycorrhizal (*Glomus intraradices*) inoculation improved the growth of both the transgenic (yeast metallothionein *CUP1*) and non-transgenic tobacco and decreased Cd concentrations in shoots and root to shoot translocation. Differences were found between the two AM fungal isolates: one isolate supported more efficient phosphorus uptake and plant growth in the soil without Cd addition, while the other isolate alleviated the inhibitory effect of cadmium on plant growth (Janouskova et al. 2005). The AM root colonization of “wild” tobacco (*N. rustica* L. var. Azteca) increased markedly from 14 to 81 % with the increasing soil Zn and the mycorrhizal structures were significantly more abundant at the highest soil Zn, suggesting that Zn may be involved directly or indirectly in AM root colonization. In addition, total Zn content or Zn concentrations in shoots and roots were shown to increase as soil Zn increased in both AM and non-AM plants. The AM roots subjected to the highest soil Zn had a significant reduction by about 50 % of the total Zn content and Zn concentration compared to non-AM roots. Yet, the relative extracted Zn percentage decreased dramatically as soil Zn increased. Soil pH was significantly lower in non-AM than AM treatments at the highest soil Zn. In summary, AM plants (particularly roots) showed lower Zn content and concentration than non-AM plants (Audet and Charest 2006). In this regard, the AM fungi have a protective role for the host plant, thus playing an important role in soil-contaminant immobilization processes; and therefore are of value in phytoremediation, especially when heavy metals approach toxic levels in the soil.

Some amendments (1 or 5 % of sepiolite, zeolite, hydroxyapatite, and apatite II^{TM}) significantly reduced Cd concentration in tobacco leaves, but the effect differed between the two soils tested. In soil 1, the use of zeolite at the 1 % dose was the most efficient, reducing the average Cd concentration from 0.6 to 0.4 mg kg^{-1}. In soil 2, the 5 % hydroxyapatite treatment led to the maximal reduction in Cd concentration (50 %). There was a dose effect for some amendments in soil 2 (containing more Cd), suggesting a reduced efficiency of the amendment at the lowest addition rate. DTPA extractable Cd and Zn measured at the end of the pot experiment were correlated to the metal concentrations in tobacco leaves (Keller et al. 2005). Golia showed a high correlation between Oriental tobacco heavy metal content and DTPA-extracted heavy metal level in soils (Golia et al. 2009).

Some soil amendments (Attapulgite, Activated carbon, Organic fertilizer) were investigated on Cd and Pb immobilization and growth of tobacco. DTPA-extractable Cd and Pb in soils were reduced to different degrees by these three soil amendments compared to control treatment. Amendments in the soils reduced uptake of Cd and Pb concentrations in tobacco roots and leaves. DTPA-extractable Cd and Pb in soils were positively correlated to metal concentrations in tobacco leaves suggesting that DTPA-extractable Cd and Pb in soils may be a feasible method to estimate Cd and Pb availability to tobacco plants and the reduction in leaf Cd and Pb concentration was due to a reduction in metal availability to tobacco. The addition of amendments resulted in an increase in root length, shoot length, leaf area, leaf SPAD, and leaf dry weight, indicating the important role of soil amendments in protection against Cd and Pb toxicity. Furthermore, higher doses of amendments used resulted in better effects (Zhao et al. 2009). In general, soil amendments were effective in improving tobacco yield and quality.

AM fungi isolated from polluted soils were no more effective than those from unpolluted soils when grown in symbiosis with tobacco. No significant differences were observed in roots and stalk dry weights among all treatments. Leaves and total plant dry weights were much higher in *G. versiforme* treatment than that in control treatment. As contents in roots and stalks from mycorrhizal treatments was much lower than that from control treatment, P concentrations in tobacco were not affected by colonization, nor were stalks, leaves, and total plant P contents. Meanwhile, decreased soil pH and lower water-extractable As concentrations and higher levels of As fraction bound to well-crystallized hydrous oxides of Fe and Al were found in mycorrhizal treatments than in controls. The protective effect of mycorrhiza against plant As uptake may be associated with changes in As solubility mediated by changing soil pH (Hua et al. 2009). This research confirmed that AM fungi can play an important role in food quality and safety.

5.2 Improving Soil Phytoremediation Efficacy

Soil conditioners used to aim to determine whether maize (*Zea mays*), sunflower (*Helianthus annuus*), and tobacco (*N. tabaccum*) grown on a heavy metal contaminated soil containing copper, zinc, and cadmium could be used to gradually remediate the soil, while producing valuable biomass. The highest concentrations of Cd, Cu, and Zn occurred in the leaves and/or roots, while seeds and grains contained much lower concentrations of these elements. All these concentrations, however, were still in the ranges considered normal for the respective plant parts grown in uncontaminated soil. While sunflower and maize could be safely used as food and feed, tobacco would be better used for bioenergy than for cigarette production because of its relatively high foliar Cd concentration. The two treatments (S and NTA) had only slight effects on the uptake and allocation of plant nutrients and Cd (Fassler et al. 2010). Thus, there was little benefit of these treatments for phytoextraction purposes at this site. EDDS revealed a higher toxicity to tobacco (*N. tabacum*) in comparison to EDTA, but no toxicity to microorganisms. The uptake of Cu was increased by the addition of EDTA and EDDS, while no increase was observed in the uptake of Cd. Both chelating agents showed a very low root to shoot translocation capability and the translocation factor was lower than that of control. Contrary to previous opinions the results of this study revealed the chelating agents EDTA and EDDS as unsuitable for enhanced phytoextraction using tobacco (Evangelou et al. 2007).

Evangelou et al. (2006) investigated the use of three natural low molecular weight organic acids (NLMWOA) (citric, oxalic, and tartaric acid) as alternative to synthetic chelators for enhancing heavy metal extraction by tobacco. A significant increase in copper uptake was visible only in the citric acid treatment (67 mg kg^{-1}) in comparison to the EDTA treatment (42 mg kg^{-1}). The NLMWOA application showed no enhanced effect concerning the lead phytoextraction. A possible explanation for this lack of significance could be the rate of the degradation of NLMWOA. Thus NLMWOA was unsuitable to enhance phytoextraction of heavy metals from soil through tobacco. Three tobacco (*N. tabacum* L.) varieties, Basma BEK, K326, and TN90, were selected for the experiment. Each variety belonged to a distinct tobacco type important for commercial tobacco production, Oriental, flue-cured, and Burley (respectively). Cd concentration in leaves was decreased by inoculation with selected isolates in the K326 and TN90 variety grown in acidic soils. In contrast, it was increased by inoculation with most isolates in the Basma BEK variety grown in a basic soil with low Cd availability. Besides, plants of all three varieties had significantly higher leaf concentrations of phosphorus and nitrogen in some inoculated treatments. AM symbiosis probably affected Cd uptake of tobacco by indirect mechanisms such as stimulation of root growth or mycorrhizal plant mediated changes in chemical or biological soil properties (Janouskova et al. 2007).

6 Conclusion

Tobacco is the main commercial crop of many countries such as China, American, Brazil, Zimbabwe, and India. Cultivation of low-hazard-tobacco (*N. tabacum* L.) has been the focus of research in tobacco sciences. Recently, numerous studies indicated that cadmium accumulation by tobacco in polluted soils had become one of the main factors affecting the tobacco quality. Cadmium was readily accumulated by crops such as tobacco in soils, and the cadmium stress in soils could not only affect the physiological process, but also reduce the quality of tobacco. There was special transport, accumulation, and compartmentalization of HMs in tobacco. Antioxidative enzymes and antioxidants activity, trichomes and crystals formation, the special genes were the main physiological detoxication mechanism of tobacco to heavy metals (HMs). Some integrated approaches to reduce the uptake of HMs or improving HMs polluted soil remediation efficiency by tobacco are introduced for further study.

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